

A NEW PARASITIC COPEPOD, *ERGASILUS DANJIANGENSIS* SP. NOV.
(POECILOSTOMATOIDA, ERGASILIDAE) ON GILLS OF TWO CYPRINID
FISH *OPSARIICHTHYS BIDENS* AND *ZACCO PLATYPUS*

SONG Ying, YAO WeiJian, NIE Pin*
State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China

Abstract A new parasitic copepod, *Ergasilus danjiangensis* sp. nov. was found on gills of two cyprinid fish *Opsariichthys bidens* and *Zacco platypus* in Danjiangkou Reservoir of Hubei Province, China. Morphological examinations revealed that *E. danjiangensis* resembled the *Ergasilus* species in many characters, especially *Ergasilus briani* and *Ergasilus tumidus*, while *E. danjiangensis* can be distinguished from other *Ergasilus* species by the antenna morphology: a slippery inner edge and a cuniform like second segment with a prominent arc outer edge. Based on the internal transcribed spacer (ITS) rDNA sequences, phylogenetic study revealed that *E. danjiangensis* was much more closely related to *E. briani* than to *E. tumidus*. The morphological and phylogenetic analyses therefore suggested that *E. danjiangensis* was an independent and new species in the genus *Ergasilus*.
Key words Parasitic copepod, new species, *Ergasilus danjiangensis*, Danjiangkou Reservoir, phylogeny.

1 Introduction

Ergasilus von Nordmann, 1832 is the largest genus in the family Ergasilidae (Copepoda, Poecilostomatoida) and contains over 180 nominal species (Montú & Boxshall, 2002). To date, a total of 19 *Ergasilus* species have been reported in China (Yin, 1956; Kuang & Liu, 1991; Kuang & Qian, 1991; Liu & Wang, 1993; Zhang & Ma, 1994). In exploring fish parasites at the Danjiangkou Reservoir in Hubei Province of China, an unknown parasite was found on gills of *Opsariichthys bidens* and *Zacco platypus* with a typical copepod morphology. A previous study based on 18S and 28S rDNA sequences indicated this parasite had a relative close relationship with *Ergasilus tumidus* and *Ergasilus briani* (Song *et al.*, 2008). For a further characterization, the internal transcribed spacer (ITS) rDNA region was used to determine its phylogenetic identity. The copepod was then considered as a new species within the genus

Ergasilus.
2 Materials and Methods

2.1 Copepod collection
During the investigation into fish parasites at Danjiangkou Reservoir (32°25'-33°15' N, 110°7'-111°6' E), Hubei Province, China, a total of 52 fish species were examined and an unknown parasitic copepod was found on gills of two fish species *O. bidens* and *Z. platypus*. Female copepod adults were isolated from 55 *O. bidens* and 50 *Z. platypus* individuals. The parasites were washed with 0.7% NaCl and then preserved in 80% ethanol for further analysis. To study the relationships between this unknown copepod and other *Ergasilus* species, 4 *Ergasilus* species were also collected as well as an outgroup *Lamproglana chinensis* (Cyclopoida, Lernaeidae) (Table 1).
The geographical locations of the sample localities:

Table 1. Parasite species, fish host, geographical locality with GenBank accession numbers of the ITS sequences.

Parasite species	Fish host	Locality	GenBank No.
<i>Ergasilus tumidus</i>	<i>Acanthorhadeus taenianalis</i>	Niushan Lake	DQ328769
<i>Ergasilus danjiangensis</i>	<i>Opsariichthys bidens</i>	Danjiangkou Reservoir	DQ328770
<i>Ergasilus anchoratus</i>	<i>Pseudobagrus fulvidraco</i>	Baoan Lake	DQ328772
<i>Ergasilus hyphomesi</i>	<i>Synechogobius hasta</i>	Danjiangkou Reservoir	DQ328771
<i>Ergasilus briani</i>	<i>Misgurnus anguillicaudatus</i>	Danjiangkou Reservoir	DQ328768
<i>Lamproglana chinensis</i>	<i>Ophiocephalus argus</i>	Danjiangkou Reservoir	DQ328767

Niushan Lake (30°23'-30°29' N, 114°19'-114°29' E);
Danjiangkou Reservoir (32°25'-33°15' N, 110°7'-111°6' E);
Baoan Lake (30°12'-30°18' N, 114°39'-114°46' E).

2.2 Morphological characterization

The whole body and dissected appendages of the

copepod were sealed using 4% polyvinyl alcohol in lacto phenol with acid fuchsin (Yin, 1956). Morphological characterization was then performed under a microscope with a series of magnifications up to × 2 000.
2.3 Genomic DNA preparation and PCR amplification

This project was supported by the National Natural Science Foundation of China (30371102), and the Chinese Academy of Sciences ([2005] 192).
* Corresponding author, E-mail: pinnie@ihb.ac.cn
Received 9 Oct. 2007, accepted 5 Feb. 2008.

The genomic DNA was extracted by a standard SDS-proteinase K procedure (Sambrook *et al.*, 1989). The genomic DNA was resuspended in TE buffer (pH8.0) and stored at -20°C before being used.

The DNA fragments spanning the ITS1, 5.8S and ITS2 rDNA region were amplified using previously published primers (Luo *et al.*, 2002). The PCR reaction was performed in 100 μl mixture containing 200 ng genomic DNA, 0.2 $\mu\text{mol/L}$ primer, and 200 $\mu\text{mol/L}$ dNTP under the following conditions: 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 1 min with a final extension at 72°C for 10 min. The PCR products were gel purified and cloned into pMD 18-T vector (Takara). The cloned DNA fragments were finally sequenced with the flanking universal M13 (+) and M13 (-) primers.

2.4 Phylogenetic analyses

Sequences were aligned using Clustal X (1.83) (Thompson *et al.*, 1997), and followed by manual editing. Because the ITS2 region was too variable to be aligned, only the ITS1 and 5.8S regions were used for divergence and phylogenetic analyses. The pairwise distance corrected by Kimura two parameter model was estimated using MEGA 3.1 (Kumar *et al.*, 2004).

Phylogenetic trees for 5 *Ergasilus* species, 3 *Smeregasilus* species and 1 *Pseudergasilus* species were constructed by neighbor-joining (NJ), maximum parsimony (MP) methods (PAUP* 4.0b10; Swofford, 2003), and Bayesian Inference (BI) method (MrBayes 3.0b4; Huelsenbeck & Ronquist, 2001). The MP tree was constructed using heuristic search and 1 000 bootstrap replicates. The most appropriate evolutionary model HKY+ G selected by Modeltest 3.7 (Posada & Crandall, 1998) was employed for the NJ and BI analyses. The NJ method was also performed with 1 000 bootstrap replicates. Branches with bootstrap values of 70% or greater were regarded as sufficiently resolved (Huelsenbeck & Hillis, 1993). For Bayesian analysis, the MCMC process was set for four chains to run simultaneously for 1 000 000 generations, with trees being sampled every 100 generations. After 'burn-in' the first 1 000 trees, a majority-rule consensus tree was constructed.

The approximately unbiased (AU) test and Shimodaira-Hasegawa (SH) test, as implemented in the program Consel v0.1f (Shimodaira & Hasegawa, 2001), were used to test the significance of the difference between alternative tree topologies with the appropriate constraint enforced and the optimal tree topology.

3 Results

3.1 *Ergasilus danjiangensis* sp. nov. (Figs. 1-13, Table 2)

Type material. Holotype ♀, Hubei, Danjiangkou

Reservoir, 22 June 30 2005, collected by SONG Ying. Fifty type-specimens of *E. danjiangensis* coded as ED Jnov 2005 were preserved in 80% ethanol and deposited in the Laboratory of Fish Diseases, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei, China.

Description of adult female. Body cyclops-like. Body length 1.11-1.27 mm ($n = 10$). Cephalothorax length 0.57-0.66 mm and width 0.28-0.43 mm. First pedigerous somites incorporated into cephalothorax. 3 free posteriorly reducing (in width) pedigerous somites (i. e., second, third, and fourth somites) (Figs. 1-2). A relatively large third somite, about 1.07-1.23 times and 1.51-1.77 times the length of the second and fourth somites, respectively. A short fifth pedigerous somite visible only ventrally. Genital double-somite barrel-shaped and narrowing posteriorly; paired genital apertures oriented longitudinally on dorsal surface of the genital somite; genital somite length 0.07-0.11 mm and width 0.10-0.16 mm, about 1.48-1.72 times the length of three abdominal segments. Near identical large first and second abdominal somites, about 2 times the size of the third somite. Caudal ramus with 4 setae, the innermost being longest.

Antennule (Fig. 3) six-segmented, tapering distally; the third segment being longest and nearly square. Seta formula: 1, 7, 5, 3, 2, 5 from the first to the terminal segment.

Antenna (Figs. 4-5) five-segmented, short and stout. The coxobasis short; the second segment cuniform having an arc outer edge; terminal claw curved and slippery.

Mouthparts (Figs. 6-8), the centre of the cephalothorax, comprising mandible, maxillule, and maxilla. Maxilliped lacking. Mandible (Fig. 6) unsegmented, armed with one blade anteriorly fringed with fine feathery teeth; the horizontal base stout and long, bearing a narrow palp armed with pointed teeth. Maxillule (Fig. 7) transversely oval knobs with two stout setae. Maxilla (Fig. 8) two-segmented; broad proximal segment tapering distally; distal segment narrow, bearing several spines at the end.

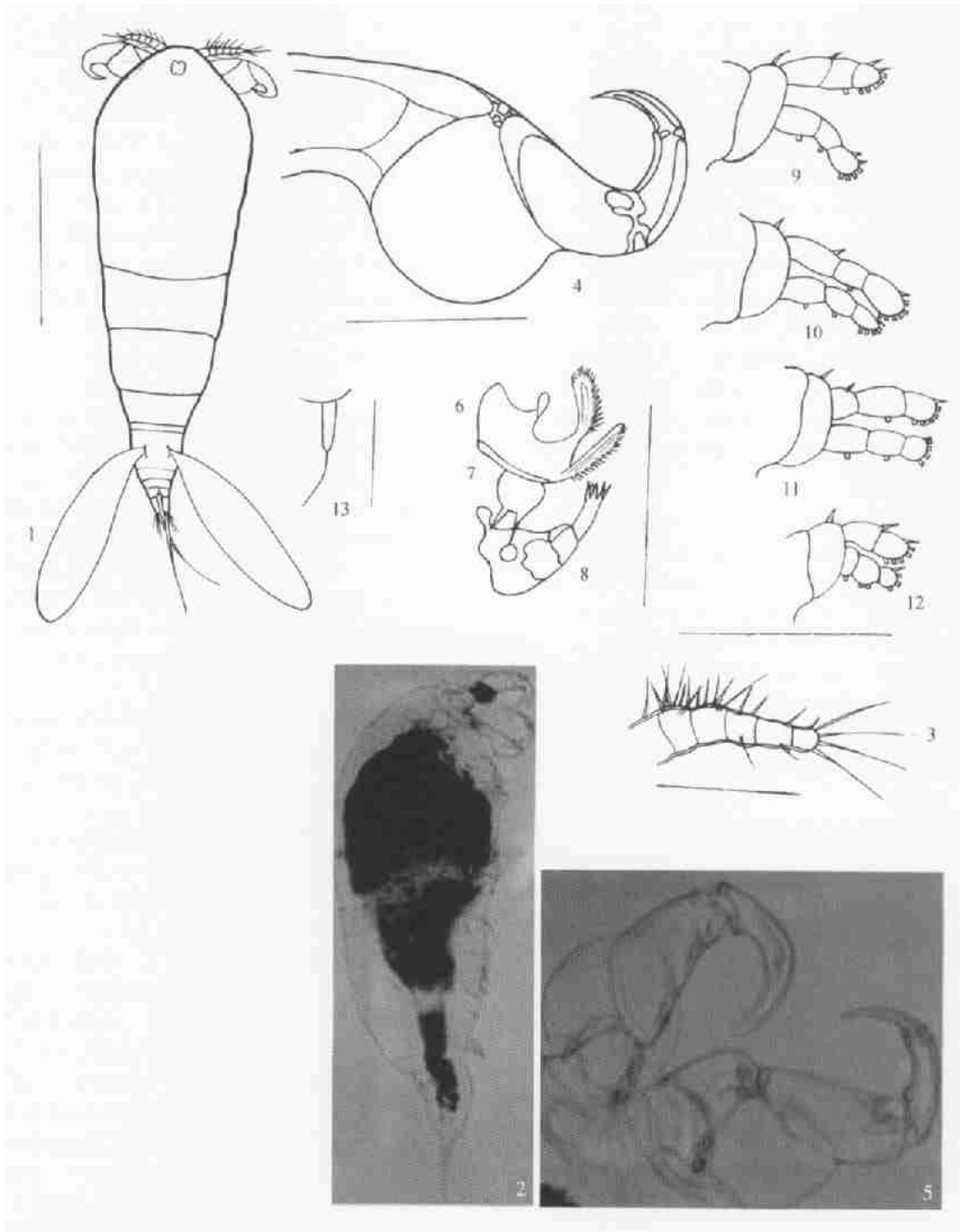
Swimming legs 1-4 biramous (Figs. 9-12) with rami three-segmented, except two-segmented exopod of leg 4 (Fig. 11). Leg 5 uniramous and reduced to a single short and narrow segment armed with a long terminal seta (Fig. 13). Spine and seta formula in Table 2.

Host. *Opsariichthys bidens* Günther, 1873 (Teleostei, Cyprinidae), *Zacco platypus* Temminck and Schlegel, 1846 (Teleostei, Cyprinidae).

Prevalence and intensity of *E. danjiangensis* on *O. bidens*: 40%; 1-55 parasitic copepods per fish.

Distribution. China (Danjiangkou City).

Etymology. The species named after the type locality.



Figs 1-13. *Ergasilus danjiangensis* sp. nov., adult female. 1. Dorsal view. 2. Lateral view. 3. Antennule. 4. Antenna. 5. Mandible. 6. Maxillule. 7. Maxilla. 8. First swimming leg. 9. Second swimming leg. 10. Third swimming leg. 11. Fourth swimming leg. 12. Fifth swimming leg. 13. Spine and seta formula.

Table 2. *Ergasilus danjiangensis* spine and seta formula.

	Coxa	Basis	Exopod	Endopod
Leg 1	0-0	I-0	I-0, 0-1, II-5	0-1, 0-1, II-4
Leg 2	0-0	I-0	I-0, 0-1, I-6	0-1, 0-1, I-4
Leg 3	0-0	I-0	I-0, 0-1, I-6	0-1, 0-1, I-4
Leg 4	0-0	I-0	I-0, I-5	0-1, 0-2, I-3

3.2 Phylogenetic analyses

The ITS DNA sequence of the 5 *Ergasilus* species, including ITS1, 5.8S, and ITS2 region, varies from 885 to 931 bp with a G + C content of 56.3%-65.1%.

Among the 5 *Ergasilus* species, the divergence level is very low between the new species *E. danjiangensis* and *Ergasilus briani* (4.6%) and *Ergasilus tumidus* (5.4%), and high between *E. danjiangensis* and *Ergasilus anchoratus* (33.1%). Sequence alignment revealed a total of 801 characters (including gaps) for 10 ITS rDNA sequences, in which 225 are variable and 138 are parsimony informative. The sequence data reported in this paper were available in the GenBank TM database under the accession numbers listed in Table 1. The other sequences

used in this study were retrieved from the GenBank™ including *Snergasilus major* (GenBank No. AY297731), *S. polycolpus* (AY297725), *S. undulatus* (AY297727), and *Pseudergasilus parasiluri* (AY297732).

All the NJ, MP, and BI trees (Fig. 14) strongly suggest that the 9 species used in this study can be divided into two groups. The first group contains 1 *Pseudergasilus* species *P. parasiluri* and 4 *Ergasilus* species including *E. tumidus*, *E. briani*, *E. danjiangensis*, and *E. hypomesi* ($BP_{NJ} = 99\%$, $BP_{MP} = 100\%$, $PP = 48\%$) and the second group contains 1 *Ergasilus* species *E. anchoratus* and 3 *Snergasilus* species including *S. major*, *S. polycolpus*, and *S. undulatus* ($BP_{NJ} = 97\%$, $BP_{MP} =$

100% , $PP = 99\%$). All the 3 trees also indicate that *E. danjiangensis* is much more closely related to *E. briani* ($BP_{NJ} = 68\%$, $BP_{MP} = 46\%$, $PP = 69\%$) than to *E. tumidus* ($BP_{NJ} = 99\%$, $BP_{MP} = 65\%$, $PP = 80\%$).

Since the closer relationship of (*E. danjiangensis* + *E. briani*) is not strongly supported by the 3 tree building methods, the closer relationship of (*E. danjiangensis* + *E. tumidus*) was then hypothesized. Hypothesis testing suggested that there was no significant differences between the two topologies according to AU ($P = 0.239$) and SH tests ($P = 0.256$). The results also indicate that the tree with the clade (*E. danjiangensis* + *E. briani*) is the best tree.

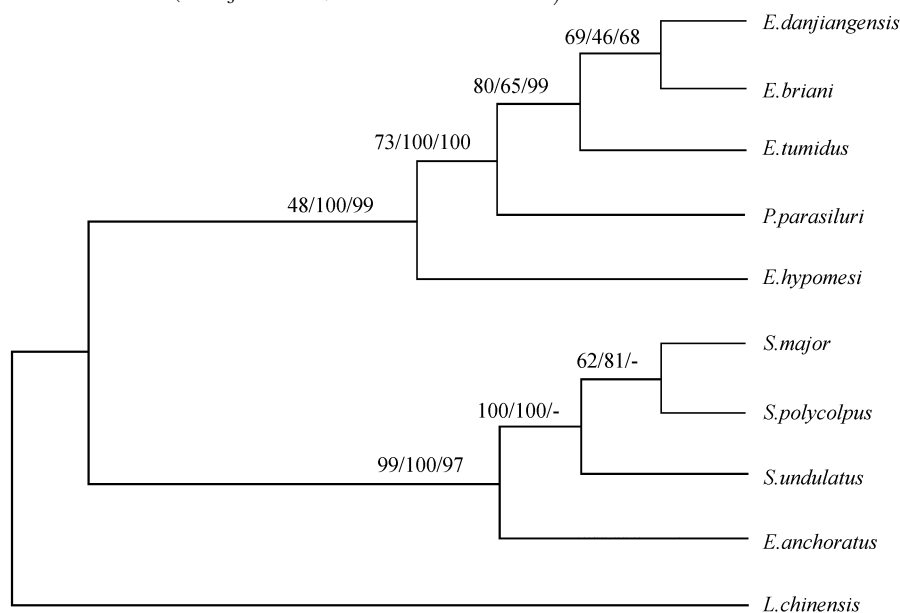


Fig. 14. Bayesian inference tree based on the ITS rDNA sequences. Numbers along branches indicate the percentages of support values resulting from different analyses in the following order: bayesian inference/ maximum parsimony/ neighbor joining. Clades not suggested by the neighbor-joining method are indicated with dashes.

4 Discussion

E. danjiangensis sp. nov. resembles many different *Ergasilus* species in different features, such as the one blade armed mandible similar to *E. briani*, *E. tumidus*, *E. glyptothoracis*, and *E. amblycephalus*, spines at the end of distal segment of maxilla similar to *E. briani* and *E. tumidus*, cunifomr like second antenna segments similar to *E. briani*, *E. tumidus*, *E. hypomesi*, *E. sieboldi*, *E. glyptothoracis*, and *E. xianjiangensis*, and slippery inner edge of the antenna similar to *E. tumidus* (Yin, 1956; Kuang & Qian, 1991). In summary, *E. danjiangensis* can be distinguished solely by the antenna morphology: a slippery inner edge and a cunifomr like second segment with a prominent arc outer edge. *E. gibbus*, though with a similar antenna structure to *E. danjiangensis*, can be characterized by the presence of a spine at the inner edge of its antenna (Fryer, 1982).

In addition, *E. danjiangensis* can be also

characterized by its strict host specificity. *E. danjiangensis* was found on only 2 of the 52 freshwater fish species, i. e. *O. bidens* and *Z. platypus* investigated in this study. So far, only *E. briani* has been reported on *O. bidens* (Yin, 1956; Kuang & Qian, 1991), but it is clearly different from *E. danjiangensis* in many morphological features, such as antennule seta formula, spine containing antenna, spine and seta on the swimming legs etc (Yin, 1956).

Morphological comparison suggests that *E. danjiangensis* resembles *E. tumidus* and *E. briani* in many characters, indicating these 3 species may have a relatively close relationship. Subsequent phylogenetic analysis and AU and SH tests using the ITS region suggest that *E. danjiangensis* is much more closely related to *E. briani* than to *E. tumidus*. Using the ITS DNA sequence, their inter species divergence level was further determined to be 4.6% between *E. danjiangensis* and *E.*

briani and 5.4% between *E. danjiangensis* and *E. tumidus*. This high divergence level, together with morphological studies, clearly suggests that *E. danjiangensis* is a newly identified *Ergasilus* species.

REFERENCES

- Fryer, G. 1982. The parasitic Copepoda and Branchiura of British freshwater fishes: A Handbook and Key. Freshwater Biological Association Scientific Publication. Ambleside, 87 p.
- Huelsenbeck, J. P. and Hillis, D. M. 1993. Success of phylogenetic methods in the four-taxon case. *Syst. Biol.*, 42: 247-264.
- Huelsenbeck, J. P. and Ronquist, F. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17: 754-755.
- Kuang P. R. and Liu, D S 1991. A new species of the family Ergasilidae and its SEM observation (Crustacea: Copepoda). *Acta Zootaxonomica Sinica*, 16: 403-406. [动物分类学报]
- Kuang P. R. and Qian, J-H 1991. Economic Fauna of China: Parasitic Crustacea of Freshwater Fishes. Science Press, Beijing. 203 p.
- Kumar, S., Tamura, K. and Nei, M. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief. Bioinformatics*, 5: 150-163.
- Liu, J-X and Wang, W-J 1993. Two new species of the family Ergasilidae from the freshwater fishes of China. *Transactions of Researches on Fish Disease*, 1: 135-140.
- Luo, H Y, Nie, P, Zhang, Y-A, Wang, G-T and Yao, W-J 2002. Molecular variation of *Bothriocephalus acheilognathi* Yamaguti, 1934 (Cestoda: Pseudophyllidea) in different fish host species based on ITS rDNA sequences. *Syst. Parasitol.*, 52: 159-166.
- Montú, M. A. and Boxshall, G. A. 2002. *Gauchergasilus*, a new genus for *Ergasilus aripalesi* Montu, 1980, an abundant parasitic copepod from the Patos Lagoon in southern Brazil. *Syst. Parasitol.*, 51: 21-28.
- Posada, D. and Crandall, K. A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics*, 14: 817-818.
- Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. Analysis and cloning of eukaryotic genomic DNA. In: Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. pp. 9, 1-9. 59.
- Shimodaira, H. and Hasegawa, M. 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics*, 17: 1246-1247.
- Swofford, D. L. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.
- Song, Y, Wang, G-T, Yao, W-J, Gao, Q and Nie, P 2008. Phylogeny of the freshwater parasitic copepods in the Ergasilidae (Copepoda: Poecilostomatoida) based on 18S and 28S rDNA sequences. *Parasitol. Res.*, 102: 299-306.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. and Higgins, D. G. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, 24: 4876-4882.
- Yin, W-Y 1956. Studies on the Ergasilidae (parasitic Copepoda) from the freshwater fishes of China. *Acta Hydrobiologica Sinica*, 2: 255-260.
- Zhang Q-Z and Ma, G-L 1994. A new species of the genus *Ergasilus* parasitizing freshwater fish in Sichuan Province, China. *Acta Zootaxonomica Sinica*, 19: 139-143. [动物分类学报]

马口鱼和宽鳍 鲮上发现寄生桡足类一新种 (杯口水蚤目, 鲮科)

宋 英 姚卫建 聂 品

中国科学院水生生物研究所 武汉 430072

摘 要 在湖北省丹江口水库的马口鱼和宽鳍 鲮的鳃上发现的寄生桡足类 1 新种丹江鲮。形态观察的结果表明, 丹江鲮与鲮属的其他种类有很多共同特征, 尤其是与博氏鲮和膨大鲮。与其它所有鲮属种类不同的是丹江鲮的第 2 触肢内缘光滑无刺, 及其楔形第 2 节的外缘呈明显的弧状。以核糖体内

关键词 寄生桡足类, 新种, 丹江鲮, 丹江口水库, 系统进化。
中图分类号 Q959.223.3

转录间隔区 (ITS) 为标记进行系统进化分析, 结果表明, 与膨大鲮相比, 博氏鲮与丹江鲮的亲缘关系更近些。无论是形态还是系统进化分析的结果都支持丹江鲮为鲮属 1 个独立的新种。